Journal of Biological Response Modifiers
4:484–489 © 1985 Raven Press, New York

Optimization of an Immunotherapeutic Protocol with Poly(I,C)-LC

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Summary: The development of successful immunotherapeutic protocols requires new therapeutic strategies as well as the development of clinically predictive tumor models and protocols. A bell-shaped response curve is observed with many biological response modifiers (BRMs), not only for immunomodulation, but also for therapeutic activity. Although most BRMs, including poly(I,C)-LC, are toxic at high doses, the administration of nontoxic doses by an optimal protocol and route of injection results in significant therapeutic benefit and increased immunodulation in tumor-bearing animals compared to the administration of a maximum tolerated dose (MTD). We suggest that Phase II clinical trials using an optimal immunomodulatory protocol may result in increased therapeutic activity compared to protocols based on the MTD. **Key Words:** Immunotherapy—Metastasis—Poly(I,C)-LC.

Because metastasis is the major threat to cancer patients, the emphasis in the therapeutic evaluation of biological response modifiers (BRMs) should be on the assessment of therapeutic efficacy against systemic lesions. Two models of immunotherapy are utilized by the Preclinical Screening Laboratory (1–6): one directed against tumor cells systemically introduced into the host by intravenous injection (experimental metastasis), and the other, a more clinically relevant model, is the assessment of activity against metastatic foci that develop after the resection of a primary tumor (spontaneous metastasis). In the spontaneous metastasis model, the host has been conditioned by tumor growth for some period of time prior to the initiation of therapy following surgical resection of the primary tumor. Therapeutic models directed against metastatic disease, in which therapy is initiated 1 or 2 days following primary tumor transplantation, represent a study of primary tumor therapy as well as a form of metastatic prophylaxis, as therapy is started prior to the tumor cell dissemination to form metastases. Furthermore, with the latter type of model, the primary tumor burden in the treated animals is often smaller than in saline-treated

Received June 11, 1985; accepted June 11, 1985.

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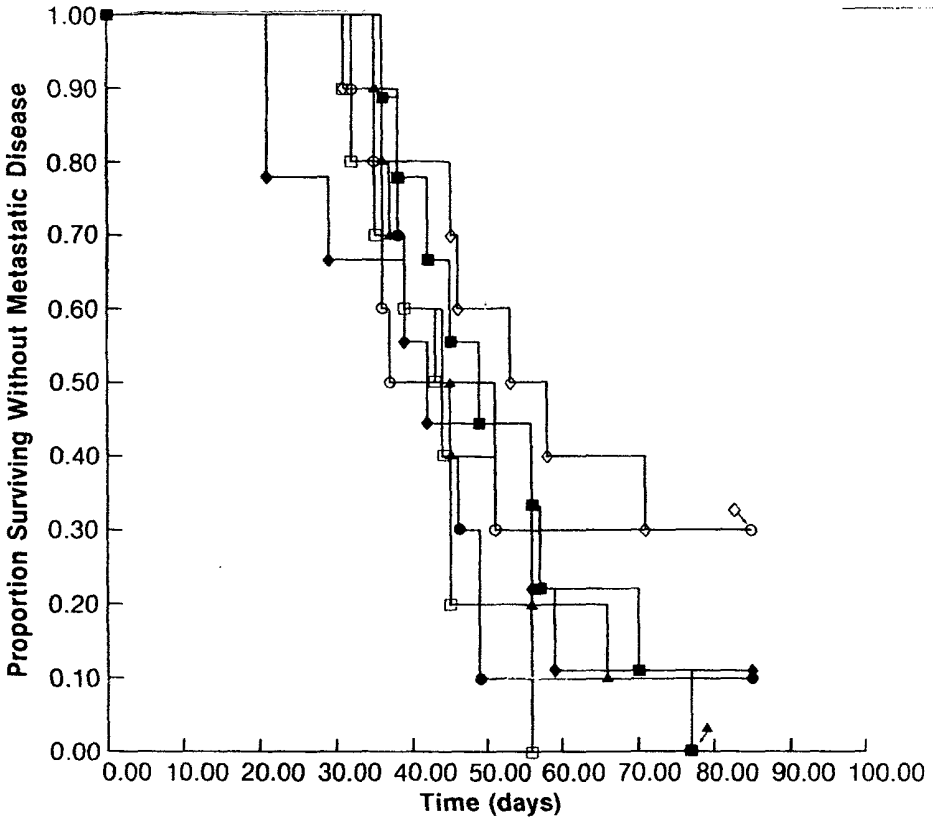

FIG. 1. Treatment of experimental metastases by the systemic administration of multilamillar vesicles incorporating MTP-PE or poly(I,C)-LC. Mice were given twice-weekly intravenous injections for 4 weeks, starting 3 days following the intravenous challenge of syngeneic mice with 25,000 B16-BL6 tumor cells (n = 10). Treatment groups included HBSS (open squares), multilamillar vesicles incorporating MTP-PE (open circles), poly(I,C)-LC at 2.5 mg/kg (filled triangles), poly(I,C)-LC at 1.2 mg/kg (filled diamonds), poly(I,C)-LC at 0.5 mg/kg (filled squares), poly(I,C)-LC at 0.05 mg/kg (open diamonds), and poly(I,C)-LC at 0.005 mg/kg (filled circles). The plots are Kaplan-Meier survival curves.

hosts, thereby reducing metastatic disease and preventing a valid comparison of metastatic burden with the control animals, which have a more extensive primary and potentially more extensive secondary tumor burden. However, even the spontaneous metastasis model utilizes young, relatively healthy hosts bearing transplantable tumors, a situation that may be quite disparate from that of cancer patients who may have been exposed to carcinogenic insult many years before and who have gradually developed progressive primary and metastatic tumor lesions over a prolonged period. Thus, we suggest that the most clinically relevant tumor model would be one with primary, autochthonous tumors that metastasize (7,8).

RESULTS

Therapy of Experimental Metastatic Foci Using Poly(I,C)-LC

The therapeutic potential of polyinosinic-polycytidylic acid solubilized with poly-L-lysine in carboxymethyl cellulose [poly(I,C)-LC] was investigated using tumor models of both experimental and spontaneous metastases (9). In the experimental metastasis model, therapy was initiated 3 days following the intravenous injection of B16-BL6 tumor cells (Fig. 1). At this time, multiple microfoci of pulmonary tumor nodules are histologically evident. Administration of poly(I,C)-LC significantly reduced the number of experimental metastases in a dose-dependent manner and significantly prolonged the survival of mice bearing experimental metastases. Poly(I,C)-LC prolonged survival at 0.05 and 0.5 mg/kg, as determined by the generalized Kruskal-Wallis analysis (0.014 and 0.047, respectively), with 0.05 mg/kg poly(I,C)-LC having significantly better therapeutic benefit in this experiment than 0.5 mg/kg poly(I,C)-LC. Furthermore, treatment of animals with preexistent experimental metastases with a 0.05 mg/kg dose of poly(I,C)-LC "cured" 30% of the animals. The treatment of mice with higher levels of poly(I,C)-LC (2.5 or 1.25 mg/kg) had significantly less therapeutic activity compared to poly(I,C)-LC at 0.05 mg/kg. In a subsequent experiment, syngeneic mice bearing UV-2237 mm pulmonary nodules were treated with poly(I,C)-LC starting either 2 or 8 days following tumor challenge (9). In this study, poly(I,C)-LC at 1, 0.5, and 0.25 mg/kg were equally effective in reducing the number of pulmonary metastases in the animals in which treatment was started 2 days following tumor challenge. In contrast, when therapy was delayed until 8 days following tumor challenge, only poly(I,C)-LC at 1 mg/kg was effective in significantly reducing the number of pulmonary metastases. The lower doses of poly(I,C)-LC were not efficacious against this increased tumor burden. Thus, it appears that although lower doses of poly(I,C)-LC may be effective against minimal tumor burden, higher doses of poly(I,C)-LC may be required in animals bearing extensive tumor burden.

Therapy of Spontaneous Metastases

The therapy of spontaneous metastases by the administration of poly(I,C)-LC was begun 4 weeks after tumor injection and 3 days following surgical resection (10). Poly(I,C)-LC administered twice weekly for 4 weeks also resulted in an immunotherapeutic response. Mice that received excipient [Hanks' balanced salt solution (HBSS)] injections developed a median of 10 spontaneous metastases (90% incidence of metastases). However, mice treated with poly(I,C)-LC at 2.5 mg/kg had a median of 0 spontaneous metastases, with 70% of the animals remaining tumor-free at necropsy. Mice treated with poly(I,C)-LC at 0.25 mg/kg had a median of 3 pulmonary metastases, with 50% of the mice being tumor-free. In a second experiment, the control animals treated with HBSS developed a median of 14 pulmonary metastases (100% incidence of metastasis), whereas mice treated with poly(I,C)-LC at 1.25 mg/kg had a median of 0 pulmonary metastases, with 55% of the mice remaining tumor-free. Thus, poly(I,C)-LC has therapeutic efficacy, not only against the limited tumor burden seen with experimental metastases, but also against well-established spontaneous metastases in a tumor-compromised host.

Scheduling of Immunotherapy With Poly(I,C)-LC

Optimal scheduling of poly(I,C)-LC immunotherapy was investigated using the experimental metastasis model in which therapy was initaited 3 days following tumor challenge. In one study to determine the optimal number of injections per week, poly(I,C)-LC at 1.25 mg/kg was administered 1, 2, and 3 times a week or daily for 4 weeks. Significant prolongation of survival was observed when poly(I,C)-LC was given 2 ($p = 0.03$) or 3 times ($p = 0.001$) per week. No significant prolongation of survival was observed in animals treated once per week as compared with animals treated with 0.9% NaCl solution (saline). The 3-times-weekly administration of poly(I,C)-LC provided significantly better protection than did the twice-weekly administration ($p = 0.005$). Furthermore, daily administration of poly(I,C)-LC was not significantly better than the 3-times-weekly administration.

To determine whether the administration of poly(I,C)-LC for 4 weeks was required, or if a shorter schedule would be sufficient, poly(I,C)-LC was delivered on a suboptimal, twice-weekly schedule for 1, 2, 3, or 4 weeks beginning 3 days following tumor challenge. No therapeutic efficacy was observed when mice were given poly(I,C)-LC twice a week for only 1 or 2 weeks; however, at 0.5 mg/kg poly(I,C)-LC, a significant decrease in the median number of metastases was observed when it was administered for 3 weeks ($p = 0.027$; Kruskal-Wallis analysis). When therapy was continued for 4 weeks (at both 0.5 and 2.5 mg/kg), it resulted in a significant prolongation of survival as determined by the Kruskal-Wallis analysis ($p = 0.0013$ and 0.0007, respectively).

The therapeutic efficacy of BRMs, including poly(I,C)-LC, against autochthonous UV-induced tumors have been monitored by the growth kinetics of the primary tumor and the prolongation of host survival (7,8). Initial therapeutic studies against UV-induced tumors demonstrated that liposomes incorporating muramyltripeptide-phosphatidylethanolamine (MTP-PE) significantly delayed the growth of UV-induced tumors, prolonged the host survival, and reduced the incidence of spontaneous metastases from 30% to approximately 10%. Recent studies on treatment of UV-induced tumors included poly(I,C)-LC, which was administered at 1 μg/animal biweekly for 12 weeks. In these studies, the administration of poly(I,C)-LC at nontoxic, optimal immunomodulatory doses (OID) significantly prolonged the survival of the animals from a median survival time (MST) of 13 weeks for the saline-injected animals to an MST of 20 weeks in the animals treated with poly(I,C)-LC ($p = 0.012$ by the Kruskal-Wallis survival analysis). It should be noted that these studies required 10 months to induce the tumor and are still ongoing 40 weeks following tumor induction (i.e., a complete study requires approximately 2 years).

One unresolved question regarding immunotherapy is whether or not immunomonitoring studies can be utilized to determine (individually, if necessary) the optimal therapeutic protocol (OTP) for an immunomodulator. Therefore, studies were undertaken to determine if there is a correlation between the survival of tumor-bearing animals and the augmentation of specific effector cell activities. As shown in Table 1, we examined the therapeutic activity of poly(I,C)-LC using the optimal immunomodulatory dose for normal mice (10 μg/animal, administered daily), the maximum tolerated dose (50 μg/animal, administered daily), and the optimal therapeutic protocol (100 μg/animal, administered biweekly). The therapeutic model utilized MBL-2 tumor ascites, and therapy was initiated 48 h following the intraperitoneal injection

TABLE 1. *Effector cells correlation between host survival and function in the treatment of MBL-2 ascites tumor-bearing mice*[a]

Treatment	MST	% Macrophages in the ascites	Macrophage cytotoxicity	CTL MBL-2	NK cell activity YAC
PBS	20	1	0	3	0
Poly(I,C)-LC 10 μg/animal[b]	36	65	10	10	10
Poly(I,C)-LC 50 μg/animal[b]	34	74	11	13	16
Poly(I,C)-LC 100 μg/animal[c]	43	75	16	17	25
Statistical analysis[d]	—	p = 0.05	p = 0.01	p = 0.05	p = 0.08

CTL, cytotoxic T lymphocyte activity; NK, natural killer; MST, median survival time.
[a]Syngeneic mice were injected intraperitoneally with 50,000 MBL-2 ascites cells, and 2 days later, therapy was initiated by intraperitoneal administration.
[b]qd, Daily administration.
[c]biw, Biweekly.
[d]Pearson's correlation of medians.

of 50,000 MBL-2 ascites cells. The functional activity of tumor ascites-associated macrophages was determined in a 72-h assay of macrophage tumoricidal activity using [^{125}I]IUdR-labeled B16-BL6 cells (10), and host spleen cell activity was studied as directed against tumor-specific targets (MBL-2) or nonspecific targets (P815) (10), and natural killer (NK) cell activity using YAC-1 target cells (10). The splenic cytotoxic activities were determined in a 4-h chromium release assay (10). Using the statistical analysis of Pearson's correlation for medians, we found that there was a direct correlation between immunotherapeutic activity and the percentage of macrophages in the tumor ascites ($p = 0.05$), macrophage cytotoxic function ($p = 0.01$), cytotoxic T lymphocyte (CTL) activity against specific targets ($p = 0.05$) and nonspecific P815 targets (data not shown, $p = 0.04$), and a nearly significant correlation with NK cell activity ($p = 0.08$). These results encourage the incorporation of immunomonitoring studies during clinical trials and suggest that they may be useful in developing therapeutic protocols for subsequent clinical trials.

DISCUSSION

The development of successful immunotherapeutic protocols requires new therapeutic strategies, as well as the development of clinically predictive tumor models and protocols. Beyond the determination of a maximum tolerated dose (MTD), it is also important to determine the OID, not only in animal models, but also clinically. Furthermore, it should be determined if either the MTD or OID represents the optimal therapeutic dose (OTD). The OTD appears to be dependent not only on the augmentation of effector cell activity, but also on the number, function, and tumor infiltration of effector cells. The determination of an optimal therapeutic protocol (OTP) requires additional information on an optimal therapeutic schedule and duration of treatment. Studies of scheduling parameters have revealed that many BRMs induce host toxicity when delivered frequently at high doses. However, if the BRM is administered at either a lower level or less frequently, there can be a decrease in toxicity that may be paralleled by greater therapeutic activity. Thus, in agreement

with studies of in vitro and in vivo immunomodulation, a bell-shaped dose–response curve for therapeutic activity may be observed. This could be due to decreased immunomodulatory potential at higher BRM doses or it may be associated with host toxicity which, concomitant with the tumor burden, may result in morbidity or decreased survival as compared to an OTP.

In summary, poly(I,C)-LC appears to have significant therapeutic efficacy at nontoxic doses when administered using an OTP, which also was found, at least within the MBL-2 tumor ascites models, to be the optimal immunomodulatory protocol. We suggest that this BRM has clinical–therapeutic potential when administered using an approach to determine the optimal immunomodulatory protocol.

Acknowledgment: This research was sponsored by the National Cancer Institute, DHHS, under Contract N-1-23910 with Program Resources, Inc.

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